NOTE TO FILE (BNF0030)

Keywords:

Cotton, Gossypium hirsutum, acetolactate synthase (ALS), sulfonylurea herbicide tolerance, tobacco, Nicotiana tabacum cv. Xanthi.

Background

In a submission dated February 23, 1996, DuPont provided summary information to support their safety assessment of cotton (Gossypium hirsutum) line 19-51a.

Intended Effect and Food/Feed Use

According to DuPont, cotton line 19-51a is genetically modified to express a chimeric gene, designated the chimeric S4-HrA gene, which encodes a sulfonylurea herbicide tolerant form of the enzyme acetolactate synthase (ALS). The chimeric gene was originally derived from two different tobacco (Nicotiana tabacum cv. Xanthi) ALS genes that both encoded herbicide sensitive versions of ALS. DuPont introduced two resistance mutations into one of these ALS genes by site-directed mutagenesis. A DNA fragment containing these two mutations was moved into the second ALS gene by employing a common restriction enzyme fragment. These genetic manipulations by DuPont led to the development of the herbicide tolerant form of ALS encoded by the chimeric S4-HrA gene, which was inserted into cotton cultivar Coker 312, producing the cotton line 19-51a.

Sulfonylurea herbicides control weeds by inhibiting the ALS enzyme, which catalyzes the common initial step in the biosynthesis of the essential amino acids isoleucine, leucine, and valine.

Cotton is primarily used as a source of textile fiber. Cotton linters, cottonseed oil, and cottonseed meal are by-products that are used in human and animal foods. Cotton linters are used as a source of cellulose in food for human consumption. Cottonseed oil is commonly used as a vegetable oil in human food. Cottonseed meal is used primarily in animal feed as a source of protein.

Molecular Alterations and Characterization

The chimeric S4-HrA gene present in 19-51a expresses an herbicide tolerant ALS, which allows the cotton plant to synthesize essential

amino acids in the presence of sulfonylurea herbicides. The chimeric gene was originally derived from two different tobacco (Nicotiana tabacum cv. Xanthi) ALS genes that both encoded herbicide sensitive versions of ALS.

According to DuPont, sulfonylurea herbicide tolerant tobacco cell lines were selected and plants were regenerated, with several of the latter displaying up to 100-fold increases in tolerance to foliar application of sulfonylurea herbicide. Tolerance in these tobacco lines was attributed to dominant nuclear mutations at either of two unlinked chromosomal loci. Tobacco lines derived from plants homozygous for one of the mutations were further selected using a higher herbicide concentration, and one tolerant line was selected. Increased tolerance was determined by DuPont to be causal to a second mutation linked to the first. Subsequently, tobacco plants homozygous for both mutations showed at least a 1000-fold increase in tolerance to sulfonylurea herbicide as compared to non-variant parental genotype plants. DuPont determined that tolerance in these test tobacco plant mutants was related to the production of an herbicide insensitive form of the ALS enzyme.

The ALS genes from the single and double mutant tobacco lines were isolated by creating genomic libraries in bacteriophage lambda, which were screened with a wild-type tobacco ALS gene probe. The locations of the ALS genes in the positive phage clones were, then, restriction enzyme mapped, revealing two loci designated ALS 1 and ALS 2. DNA sequence analysis of the ALS genes encoding sulfonylurea-sensitive and -resistant phenotypes further revealed the mutation sites. DuPont attributed ALS resistance to two amino acid changes in the protein sequence.

The chimeric S4-HrA gene was constructed by DuPont by combining fragments of DNA from the ALS 1 and ALS 2 genes. Mutations similar to those in the double mutant tobacco line were introduced into the ALS 2 sensitive gene by site-directed mutagenesis. A restriction fragment containing the two introduced mutations was subcloned into the ALS 1 sensitive gene.

The chimeric S4-HrA gene was transferred into cotton by DuPont, using the binary vector based Agrobacterium tumefaciens plant transformation system. The plasmid used by DuPont for cotton transformation was designated pMH26, which was constructed by cloning the sulfonylurea tolerant gene into the binary plasmid pZH1. The sulfonylurea tolerance gene was inserted into the pUC19 polylinker between the T-DNA borders of pZH1, which gave rise to pMH26. The pMH26 vector was replicated in Escherichia coli and

transformed into A. tumefaciens (disarmed strain LBA4404) by bacterial conjugation. The T-DNA on plasmid pMH26 was then introduced into cotton cultivar Coker 312 by inoculation of seedlings with the Agrobacterium strain containing pMH26, leading to the selection of the sulfonylurea tolerant transgenic line 19-51a.

According to DuPont, "the T-DNA introduced into cotton [cultivar Coker 312], other than the ALS gene, contains no other intact prokaryotic or eukaryotic coding sequences. Polymerase chain reaction (PCR) analyses confirmed that the chimeric S4-HrA gene was stably integrated into the cotton genome and transmitted through normal sexual reproduction. Southern blot analyses indicated that two copies of the gene, in tandem repeat, [had been] introduced at one locus and that DNA beyond the left and right borders had not been introduced into the cotton." Field testing further demonstrated that the sulfonylurea herbicide tolerance trait was stably integrated into cotton line 19-51a and is inherited as a dominant Mendelian trait.

Expressed Protein

According to DuPont, the expressed ALS protein is unlikely to be found in oil or processed meal. Moreover, ALS enzymes are found in all plants and have been isolated from some bacteria and fungi. Amino acid sequence homology amongst plant ALS enzymes is highly conserved. Therefore, humans and mammals consuming plant food would be exposed to a wide variety of very similar ALS enzymes. Mammals do not contain ALS, which DuPont theorizes may explain the low mammalian toxicity to sulfonylurea herbicides. DuPont stated that the food products derived from cotton and the ALS enzyme have not been shown to be toxic or allergenic.

Nutritional Assessment

DuPont conducted compositional analyses on transgenic cotton line 19-51a and the non-transgenic Coker 312 to determine if the concentration and bioavailability of important nutrients in the new variety were within ranges normally seen in the host species. DuPont states that the levels of protein and oil, present in seed, were essentially identical for line 19-51a and Coker 312. Levels of amino acids in transgenic cottonseed meal were not significantly different statistically, except for glutamate and aspartate, where the differences were "quite small." Moreover, DuPont emphasized that no statistically significant differences were observed for any of the essential amino acids. Cottonseed oil fatty acid levels

differed significantly (statistically) for myristic, linoleic and linolenic acids, but absolute differences were small, and "all values for both lines [fell] within the ranges adopted by the FAO/WHO Codex."

DuPont noted that "[t]wo benchmark types of components traditionally analyzed for in safety determinations of genetically modified cotton plants are cyclopropene fatty acids and gossypol." Levels of free and total gossypol in line 19-51a seeds were significantly higher than those present in Coker 312. DuPont indicates, however, that the gossypol content of both lines fell within ranges reported in the literature. Amounts of the cyclopropene fatty acids did not differ in oil obtained from the transgenic and non-transgenic lines. DuPont concluded that toxicant levels fell within the ranges normally found in commercial cotton lines.

Conclusions

DuPont has concluded that cotton line 19-51a is not materially different in composition, nutrition, and safety from cotton currently grown, marketed, and consumed for human food. At this time, based on DuPont's description of its data and analyses, the Agency considers DuPont's consultation on the aforementioned genetically modified cotton transformation event to be complete.

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